

REPUBLIC OF THE PHILIPPINES

EDICT OF GOVERNMENT

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PNS/BFAD 21 (2009) (English): Recommended Code of Practice for the Processing and Handling of Sugar Cane Wine (Basi)



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PHILIPPINE NATIONAL STANDARD

PNS/BFAD 21:2009
ICS 67.160.10

**Recommended code of practice for the
processing and handling of sugar cane wine
(basi)**

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BUREAU OF PRODUCT STANDARDS

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Table of Contents	Page
Foreword	i
1 Scope	1
2 References	1
3 Definition of terms	1
4 Raw materials, ingredients, and packaging material requirements	4
5 Hygiene	5
6 Preparation and processing of sugar cane wine (basi)	6
7 Food additives	8
8 Post-process handling procedures	9
9 Inspection and labeling	9
10 Quality assurance	9
11 Storage and transport of finished product	10
12 Laboratory control procedures	10
13 End product specification	11

Table 1 Food Additives for Sugar Cane Wine (Basi) (Codex. Stan. 192-1995 Codex General Standard for Food Additives)	8
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Annexes

A Sugar Cane Varieties	12
B Standard Method of Detection and Values for Microbiological Quality	14
C Measurement of pH of Wines	15
D Determination of Volatile Acidity	16
E Determination of Titratable Acidity in Wines	17
F Determination of Total Soluble Solids	18
G Determination of Alcohol by Volume from Specific Gravity	19
H Determination of Total Phenols	20

Foreword

This Philippine National Standard Recommended code of practice for the processing and handling of sugar cane wine (basi) was prepared by the Technical Working Group under the Phase II Project for the Development of Standards for Selected Ethnic Food Products and was endorsed for adoption as Philippine National Standard by the Bureau of Food and Drugs.

In the development of this standard different sugar cane wine samples were analyzed. Public consultation workshop was conducted in San Fernando, La Union where the product is commonly produced.

This standard was developed simultaneously with PNS/BFAD 20:2009 to serve as guide for the assurance of safety and quality and to make the locally produced sugar cane wines acceptable and globally competitive.

**Recommended code of practice for the processing and handling
of sugar cane wine (*basi*)**

1 Scope

This Code of Practice is concerned with the receipt of raw materials and ingredients, preparation and processing of sugar cane wine (*basi*) as defined in this Code, in order to conform to the required standards stated in PNS/BFAD 20:2009, Standards for sugar cane wine (*Basi*). The product may be prepared by fermentation of the juice and/or products of different varieties of sugar cane listed in, but not limited to, Annex A.

This Code is intended to provide guidelines to achieve compliance with the standard for sugar cane wine (*basi*) packed in any suitable containers.

2 References

The titles of the standards publications referred to in this standard are listed on the inside back cover.

3 Definition of terms

For the purpose of this Code, the following definitions apply:

3.1**adjunct**

it is a plant-derived product added to alcoholic beverages to contribute to their flavor and color

3.2**aging**

it is storing of wine in a sealed container after fermentation to improve its quality

3.3**brix**

it is the concentration of sugar in syrup corresponding approximately to concentration of solutes expressed in percentage as measured with a refractometer or hydrometer and expressed in Brix units

3.4**container**

it is any form of packaging material, which completely or partially encloses the food (including wrappers). A container may enclose the food as a single item or several units of types of prepackaged food when such is present for sale to the consumer

3.5

contaminant

it is any biological or chemical agent, foreign matter, or other substances that are not intentionally added to food, which may compromise food safety and suitability

3.6

current good manufacturing practices (cGMP)

it is a quality assurance system aimed at ensuring that products are consistently manufactured, packed or repacked or held to a quality appropriate for the intended use. It is thus concerned with both manufacturing and quality control procedures

3.7

ethanol

it is light, volatile alcohol produced during fermentation of sugars

3.8

fermentation

it is a metabolic process of converting reducing sugars into ethanol by yeast (*Saccharomyces* spp.)

3.9

food

it is any substance, whether semi-processed or raw, which is intended for human consumption, and includes drink, chewing gum and any substance which has been used in the manufacture, preparation or treatment of "food" but does not include cosmetics or tobacco or substances only used as drugs

3.10

food additives

It is any substance the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food (including substance intended for use in the producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding of food; and including any source of radiation intended for any such use), if such substance is generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown scientific procedures to be safe under the conditions of intended use

3.11

food standard

it is a regulatory guideline that defines the identity of a given food product (i.e. its name and the ingredients used for its preparation) and specifies the minimum quality factors and when necessary, the required fill of the container. It may also include specific labeling requirements other than or in addition to the labeling requirements generally applicable to all prepackaged foods

3.12

ingredient

It is any substance including food additive, used as a component in the manufacture or preparation of a food and present in the final product in its original or modified form

3.13

label

It includes any tag, brand, mark, pictorial or other descriptive script, written, printed, marked, embossed or impressed on, or attached to the container

3.14

lot

It is the food produced during a period of time and under more or less the same manufacturing condition indicated by a specific code

3.15

pasteurization

It is a heat treatment process applied to a product with the aim of avoiding public health hazards arising from pathogenic microorganisms. Pasteurization, as a heat treatment process, is intended to result in only minimal chemical, physical and sensory changes

3.16

packaging

it is the process of packing that is part of the production cycle applied to a bulk product to obtain a finished product. Any material, including printed material, employed in the packaging of a product including any outer packaging used for transportation of shipment. Packaging materials are referred to as primary or secondary according to whether or not they are intended to be in direct contact with the product

3.17

pH

it is the intensity or degree of acidity of a food material

3.18

phenols

these are weakly acidic organic compounds that contribute to the color, astringency and bitter flavor of wines

3.19

processing aids

these are additives that are used in the processing of food to achieve a specific technological purpose and which may or may not result in the presence of residues or derivatives in the final product (BFAD A.O. No. 88-A s. 1984)

3.20**potable water**

it is the water fit for human consumption and potability determined by health authorities cited in Philippine National Standards for drinking water (Department of Health-Administrative Order No. 2007-0012: Philippine National Standards for Drinking Water 2007)

3.21**refractometer**

it is the instrument used to measure the percent soluble solids of sugars referred to as degree Brix (°Bx); concentration of sugars expressed in terms of number of grains of sucrose per 100g of liquid

3.22**titratable acidity**

it is the amount of organic acids derived from the raw materials or produced during alcoholic fermentation, and expressed as grams of predominant acid per 100 mL of sample

3.23**volatile acids**

it is the steam-distillable acids present in the wine which is attributed to the growth of acetic acid bacteria and sometimes of yeasts; used as an indicator of spoilage and expressed as grams acetic acid per 100 mL of sample

3.24**wine**

it is an alcoholic beverage produced by the natural fermentation of the juice of grapes or other fruits or of the fermentable parts of plant or plant-related products; it contains 7 % to 24 % alcohol by volume and may contain certain optional ingredients

4 Raw materials, ingredients, and packaging material requirements**4.1 Raw materials and ingredients.**

Raw materials for processing shall not contain parasites, microorganisms, toxins, and decomposed or extraneous substances.

- a) **Sugar cane juice** – Sugar cane juice to be used for processing shall be prepared from a sound, clean and mature sugar cane variety and/or sugar cane products (such as refined and/or *muscovado* sugar) that is of a quality fit to be sold for human consumption.
- b) **Inoculum** – The inoculum to be used to facilitate fermentation of sugar cane juice shall consist of yeast cells belonging to genus *Saccharomyces* and may include other fermenting microorganisms. It could be in the form of pure cultures, active dry cells or part of the natural flora of dried adjuncts. It must not include substances and/or microorganisms that may inhibit fermentation or bring undesirable sensory and quality characteristics to the product.

- c) **Water** – Only clean, potable water (Annex B) shall be used for the preparation and for all the pretreatment and processing steps of sugar cane wine (*basi*) production. Non-potable water may be used only for operations not in direct contact with the food materials provided that this does not pose a hazard to health as determined and approved by the official agency having the jurisdiction over it.
- d) **Food additives** – All additives shall conform to the food standards required by the BFAD and/or authority.
- e) **Optional ingredients** – Adjuncts may be added during processing to achieve certain sensory characteristics for sugar cane wine (*basi*). These may be, but are not limited to samak (*Macaranga tanarius*) bark, leaves and fruits, green guava (*Psidium guajava* L.) leaves, black plum/duhat (*Syzgium jambolanum*) bark, and ginger. Only those which do not post a hazard to health may be added to the product.

4.2 Packaging materials.

The packaging materials should be appropriate for the product to be packed and for the expected conditions of handling during distribution and storage. These must provide the products adequate protection from contamination and must be sufficiently durable to withstand mechanical, chemical and thermal stresses encountered during processing and normal distribution. All packaging materials must be clean and free from defects that may affect the product or package integrity. These shall be stored in a clean and sanitary manner.

Before filling, rigid containers shall be cleaned to prevent incorporation of foreign matter into the finished product. Closures may be cleaned before use, subject to the conditions of handling by the processors or suppliers.

Glass bottles and metal closures (caps or corks) – Only heat resistant glass bottles and metal closures shall be used. The glass bottles shall be properly inspected for presence of cracks, chips and other defects. These must be washed with clean water to eliminate dirt and foreign matter.

Closures must be free from scratches, dents and other defects. It must effect a hermetic seal after processing.

Glass bottles may be reused provided they are sound, and properly washed and sanitized. All metal closures shall never be re-used. Shrinkable plastic cap seals, when used, should fit the size of the closures and glass bottles, to prevent tampering and to provide protection from bottleneck contamination and other physical damage.

5 Hygiene

It is recommended that the product covered by the provisions of this code of practice be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practice – General Principles of Food Hygiene (CAC/RCP 1 – 1969, Rev 4 (2003)) and/or the BFAD A.O. No. 153 s. 2004 – Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing,

Repacking or Holding Food, covering the plant facilities and operations requirement including the construction and layout of processing plant, hygienic facilities, equipment, utensils and working surfaces.

6 Preparation and processing of sugar cane wine (*basi*)

6.1 Preparation of raw materials for sugar cane wine (*basi*) production

a) Sugar cane juice extracted from sugar cane stalks

- (1) **Receipt of raw materials** – The sugar cane stalks shall only be accepted if it is sound and suitable for processing. Those that show signs of deterioration shall not be used.
- (2) **Inspection and sorting** – Prior to processing, the sugar cane stalks shall be inspected and sorted. The stalks shall be cut clean of leaves and tops. Sorting may be carried out on moving inspection belts or sorting tables. The stalks which suitable for sugar cane juice extraction should be separated from those suitable for vinegar.
- (3) **Washing and/or cleaning** – Sugar cane stalks are washed to remove dust, dirt, insect, mold spores, plant parts and filth that might contaminate or affect its aroma or flavor. To be most effective and economical, washing with water must be accompanied with brushing, rubbing and forcing the water against the stalk and into crevices. Detergents are frequently used in the wash or rinse water.
- (4) **Juice extraction** – Extraction of juice from sugar cane stalks should not be done more than a day after harvest. Sugar cane stalks shall be weighed, and then crushed using a crusher with stainless steel rollers. The juice shall be filtered to eliminate solid particles. It shall then be boiled for at least 30 minutes to become sterilized and more concentrated. The initial total soluble solids of the sugar cane juice shall be determined with the use of a refractometer and should not be less than 18 °Bx. Boiling of juice and/or further addition of raw material may be done to achieve the required total soluble solids. The final volume of the juice shall be measured.

b) Sugar cane juice from processed sugar cane products

- (1) **Receipt of raw materials** – The sugar cane products to be used (e.g. refined sugar, *muscovado*) shall only be those which are found suitable for human consumption. Those that show signs of deterioration shall not be used.
- (2) **Juice preparation** – The sugar cane products to be used shall be dissolved with the corresponding amount of water. Only potable water shall be used. The prepared juice shall be filtered to eliminate solid particles. The initial total soluble solids of the sugar cane juice shall be determined with the use of a refractometer and should not be less than 18 °Bx. Boiling of juice and/or further addition of raw material may be done to achieve the required total soluble solids. The final volume of the juice shall be measured.

6.2 Addition of optional ingredients

The addition of optional ingredients to achieve certain sensory characteristics for sugar cane wine (*basi*) may be done either during or after the preparation of sugar cane juice (before fermentation).

6.3 Fermentation

The sugar cane juice shall be transferred to sterilized stainless steel, glass or earthenware fermentation containers. The sugar cane juice must have been cooled up to at least 40 °C to 45 °C before addition of inoculum. Fermentation containers will be covered with a clean cloth to keep away from insects and as exhaust for carbon dioxide produced during the fermentation process. The beverage shall be allowed to ferment for at least one month, or until desired alcohol content has been reached. The minimum duration of fermentation is also to allow spent microbial cells and other sediments to settle at the bottom of the container.

6.4 Filtration

The fermented wine must be filtered of spent microbial cells and sediments using a sanitized filter machine to clarify the wine and to halt fermentation.

6.5 Pasteurization

The filtered fermented wine may be pasteurized at 70 °C for 30 minutes or any time-temperature combination to inactivate spoilage microorganisms.

6.6 Aging

The wine shall be transferred to properly sanitized glass, stainless steel or earthenware containers for aging. Containers should be tightly closed to avoid contamination and aeration. Racking, which is decanting the clear wine to another container shall be done during aging for clarification of the beverage. Racking may be done periodically until a clear wine with no sediments will be obtained.

6.7 Filling of containers

The filling of containers, either mechanically or manually, shall be controlled so as to meet the filling and headspace requirements specified in the process. It is important to standardize filling, not only for economic reasons, but because the container integrity may be affected by excessive fill variation. Properly filled containers must result in net volume equivalent to at least 90% of the water capacity of the container. Overfilling can lead to contamination of seals which can affect container integrity.

6.8 Closing and sealing of containers

Closures shall be sealed air-tight to meet the requirements of the processors. Self-sealing metal caps or lids shall be tightened and secured to each filled container.

To prevent leakage and contamination, the sealing surface shall be free of defects and damage. After closing, the caps must be essentially level, not cocked or tilted, and seated well down the finish. This will prevent damage caused by bumping of adjacent containers as they move along conveyors.

6.9 Coding of sealed containers

Coding of sealed container shall be indelible with details of production date and time, batch code, product code, the product line in which product is packed, the manufacturing plant and other information necessary for product traceability. Where the container does not permit the code to be embossed or inked, the label shall be legibly perforated or otherwise marked, and securely affixed to the product container.

6.10 Washing of sealed containers

Where necessary, filled and sealed containers shall be thoroughly washed to remove grease, dirt and product from the outside of the container.

7 Food additives

Food additives when used shall be in accordance with the regulations established by the Bureau of Food and Drugs (BFAD) (Bureau Circular No. 016 s.2006. Updated List of Food Additives), the Codex Alimentarius Commission and/or authority for these products.

The following food additives listed in, but not limited to, Table 1, may be used for the manufacture of sugar cane wine (*basi*):

**Table 1 – Food Additives for Sugar Cane Wine (*Basi*)
(Codex Stan 192-1995. Codex General Standard for Food Additives).***

Function	Additive	Maximum level
Color	Brilliant blue FCF	200 mg/kg
	Caramel III – Ammonia process	GMP
	Caramel IV – Sulphite ammonia process	GMP
	Carmines	200 mg/kg
	Carotenes, Beta-(Vegetable)	600 mg/kg
	Riboflavins	300 mg/kg
Preservative	Benzoates	1000 mg/kg (as benzoic acid)
	Dimethyl dicarbonate	250 mg/kg (added level; residue not detected in ready-to-eat food)
Antioxidant, Bleaching agent	Sulphites	200 mg/kg (as residual SO ₂)
Emulsifier, Sequestrant, Stabilizer	Diacetyltartaric and Fatty Acid Esters of Glycerol	5000 mg/kg
*Based on the food category system: 14.2.4 Wines (Other than grape).		

8 Post-process handling procedures

To control post-process leakage contamination or leaker infection, processed containers shall be dried as soon as possible after processing so that exposure to post-wet conveying and handling equipment is minimized.

9 Inspection and labeling

9.1 Inspection of finished products

All processed products shall be inspected before labeling and casing and defective products shall be withdrawn or rejected. The company must have an approved policy and procedures based on the BFAD A.O. No. 153 s. 2004 - Guidelines, Current Good Manufacturing Practices in Manufacturing, Packing, Repacking or Holding Food.

9.2 Labeling

Labeling shall be done after the prescribed incubation period when the product has passed quality evaluation. All containers shall be properly labeled. The label shall conform to the rules and regulations of BFAD (A.O. 88-B s. 1984).

9.3 Tamper-evident seals

Use of tamper-evident seal is highly recommended.

10 Quality assurance

10.1 Record keeping

Permanent and legible dated records of time, temperature code mark and other pertinent details shall be kept concerning each load. Such records are essential as a check on processing operations.

Written records of all container closure examinations shall specify the code lot, the date and time of container closure inspections, the measurements obtained and all the corrective actions taken.

Records shall be maintained identifying initial distribution of the finished product to facilitate, if necessary, the segregation of specific food lots that may have been contaminated or otherwise unfit for intended use.

All process deviations involving failure to satisfy the minimum requirements of the process shall be recorded detailing those deviations and the actions taken.

10.2 Deviations in processing

Whenever in-process monitoring records disclose that a product has a deviation from the process, the processor shall:

- i. Identify, isolate and then reprocess that portion of the production involved. Complete reprocessing records shall be retained; or
- ii. Set aside that portion of the product involved for further evaluation as to any potential public health significance. Such evaluation shall be made by competent authority and shall be in accordance with recognized procedures. A record shall be made of the evaluations made and the results. After the determination that no significant potential for health hazards exists, that portion of the product involved may be distributed. Otherwise, that portion of the product shall be destroyed.

All process deviations involving failure to satisfy the minimum requirements of the process shall be recorded detailing those deviations and the actions taken.

10.3 Good manufacturing practices (GMP)

The processing establishments shall develop and implement programs based on BFAD's Current Good Manufacturing Practices (cGMP) and Hygiene Control.

11 Storage and transport of finished product

Storage and transport conditions of the finished product shall be such that the integrity of the product container, and the safety and quality of the product are not adversely affected.

Cases and cartons must be thoroughly dry. They must be of proper size so that the containers fit snugly and are not subject to damage from movement within the case. They must be strong enough to withstand normal transport.

Extreme temperature fluctuations during storage and transport of the product must be avoided to prevent product deterioration.

12 Laboratory control procedures

Each food processing establishment shall have access to laboratory control of both the processes used and the finished products. All food ingredients and food products declared unfit for human consumption by the laboratory shall be rejected.

Representative samples for each lot or batch shall be taken to assess the safety and quality of the product.

Microbiological laboratory shall be separated from the processing area. No pathogens shall be handled within the premises of manufacturing plant.

Laboratory procedures for quality control of the processes and the product must follow recognized or standard methods for easy interpretation of results.

13 End product specifications

Appropriate methods shall be used for sampling analysis and determinations to meet the following specifications:

1. To the extent possible in good manufacturing practices, the products shall be free from any objectionable characteristics.
2. The product shall not contain any pathogenic organisms or any toxic substances originating from microorganisms.
3. The product shall be free from chemical pollutants in amounts which may represent hazard to health.
4. The product shall comply with the requirements set forth by the Bureau of Food and Drugs, the Fertilizer and Pesticide Authority of the Department of Agriculture, and the Codex Alimentarius Commission on Pesticide Residues and Food Additives.

Annex A
Sugar cane varieties*

Variety	Age of maturity (months)	Yield	Reaction to diseases
PHIL 56-226	10 to 12	High in tonnage and sugar content.	Resistant to leaf scorch, but susceptible to smut, downy mildew and yellow spot.
PHIL 58-260	12	High in tonnage and sugar content.	Resistant to leaf scorch, but susceptible to smut, downy mildew and yellow spot.
PHIL 62-120	10 to 12	High in tonnage with sugar content.	Highly resistant to downy mildew, resistant to smut, and leaf scorch.
PHIL 66-07	12	High in tonnage with sugar content.	Highly resistant to downy mildew, intermediate resistant to smut, and resistant to leaf scorch.
PHIL 67-23	12 to 14	Average in tonnage with high sugar content.	Resistant to smut and downy mildew, but intermediate susceptible to leaf scorch.
PHIL 72-28	12 to 14	Average in tonnage with high sugar content.	Very highly resistant to downy mildew, highly resistant to leaf scorch, but intermediate to smut.
PHIL 72-70	12 to 14	Average in tonnage with high sugar content.	Intermediate susceptible to smut and downy mildew, resistant to leaf scorch and yellow spot.
PHIL 74-64	12 to 14	Average in tonnage. High in sucrose content.	Resistant to smut, leaf scorch and mosaic virus.
PHIL 75-44	12 to 13	Average in tonnage, high in sugar content.	Highly resistant to smut, but susceptible to downy mildew.
PHIL 77-79	10 to 12	Average in tonnage, high in sugar content.	Resistant to downy mildew, average to leaf scorch and yellow spot, but susceptible to smut.
PHIL 80-13	10 to 12	High in tonnage and sugar content.	Resistant to smut, average to leaf scorch and susceptible yellow spot.
PHIL 80-93	12	Average in tonnage, high in sugar content.	Highly resistant to downy mildew and average to leaf scorch and yellow spot.
PHIL 83-61	12	Average in tonnage with average sugar content.	Resistant to smut, yellow spot and leaf scorch but moderately resistant to downy mildew.
PHIL 84-77	12	Average in tonnage with average sugar content.	Resistant to four major disease: smut, downy mildew, yellow spot and leaf scorch.
PHIL 85-83	10 to 12	High in tonnage and sugar content.	Resistant to smut, downy mildew, and leaf scorch but susceptible to yellow spot.

Table 1 (concluded)

Variety	Age of maturity (months)	Yield	Reaction to diseases
PHIL 87-15	12	High in tonnage with average sugar content.	Resistant to downy mildew, leaf scorch but intermediate susceptible to smut.
PHIL 87-27	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew, yellow spot.
PHIL 88-29	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew and leaf scorch moderate resistant to yellow spot.
PHIL 88-35	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew and leaf scorch, moderate resistant to yellow spot.
PHIL 88-39	12 to 14	High in tonnage and sugar content.	Resistant to smut and downy mildew.
PHIL 89-43	12	High in tonnage with average sugar content.	Resistant to smut, downy mildew, leaf scorch and susceptible to yellow spot.
PHIL 90-0345	12	High in tonnage and sugar content.	Resistant to smut, leaf scorch but susceptible to downy mildew.
PHIL 91-1091	12	High in tonnage and sugar content.	Resistant to smut, leaf scorch and to downy mildew.
PHIL 92-0051	12	Average in tonnage and sugar content.	Resistant to smut, leaf scorch, yellow spot and downy mildew.
PHIL 92-0577	12	Average in tonnage and sugar content.	Resistant to smut, downy mildew and leaf scorch moderate to yellow spot.
PHIL 92-0751	12	Average in tonnage and sugar content.	Resistant to smut, downy mildew, leaf scorch, yellow spot.
PHIL 93-2349	12	High in tonnage and sugar content.	Resistant to smut, downy mildew, and yellow spot; moderate to leaf scorch.
PHIL 93-3155	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch.
PHIL 93-3727	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch and yellow spot.
PHIL 93-3849	12	Average in tonnage and sugar content.	Resistant to smut and downy mildew; moderate to leaf scorch and yellow spot.
*Other varieties of sugar cane not listed above may also be used provided that they conform to standards stated herein.			

Reference:

Sugar Regulatory Administration. www.sra.gov.ph

Annex B

Standard method of detection and values for microbiological quality

Parameter	Method of determination	Value*	Units of measurements	Point of compliance
Total coliform	Multiple tube fermentation technique (MTFT)	< 1.1	MPN/ 100 mL	<ul style="list-style-type: none"> • Service reservoirs • Water treatment works • Consumer's Taps • Refilling Stations • Water Haulers • Water Vending Machine
	Chromogenic substrate test (Presence-Absence)*	Absent < 1.1	MPN/ 100 mL	
	Membrane filter (MF) technique	< 1	Total coliform colonies / 100mL	

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998

*Should be validated and approved by Department of Health

	Compliance to Total coliform			Consumer's Taps
	a) For water systems analyzing at least 40 samples per month, no more than 5 % of the monthly sample may be positive for total coliform; b) For water systems analyzing fewer than 40 samples per month, no more than one (1) sample per month may be positive for total coliform			
	At least 95 % of standard samples taken in each year from each reservoir are total coliform negative			• Service reservoirs
	No standard sample taken each month should exceed maximum allowance value specified in the above.			• Water treatment works • Refilling stations • Water haulers • Water vending machines
Fecal coliform	Multiple tube fermentation technique (MTFT)	<1.1	MPN/ 100 mL	• Service reservoirs • Water treatment works • Consumer's taps • Refilling stations • Point sources (Level I) • Water haulers • Water vending machines
	Membrane filter technique	< 1	Fecal coliform colonies / 100mL	
	Chromogenic substrate test (Presence Absence)*	<1.1	MPN/100 mL	
Heterotropic plate count	Pour Plate	< 500	CFU / mL	• Service reservoirs • Water treatment works • Consumer's taps nearest the meter • Refilling station • Water vending machines
	Spread Plate			
	Membrane Filter Technique			

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998

* Should be validated and approved by Department of Health

Annex C

Measurement of pH of wines

C.1 Preparation of potassium hydrogen tartrate buffer solution (Saturated solution at 25 °C, 0.034 M)

Add excess (ca 100 %) of $\text{KHC}_4\text{H}_4\text{O}_6$ (NIST SRM 188) to H_2O in glass-stoppered bottle or flask, and shake vigorously; few minutes of shaking is for saturation (100 mL H_2O at 25 °C dissolves ca. 0.7 g $\text{KHC}_4\text{H}_4\text{O}_6$). Adjust to 25 °C, let solid settle, and decant clear solution, or filter if necessary. Discard when mold appears. Few crystals of thymol added during preparation will retard mold growth, and will alter pH by unit. For accuracy of ± 0.01 pH unit, temperature of solution must be between 20 °C and 30 °C.

C.2 Calibration of pH meter

Let pH meter with glass and calomel electrodes warm up before use according to manufacturer's instructions. Check meter with freshly prepared, saturated, aqueous solution of $\text{KHC}_4\text{H}_4\text{O}_6$. Adjust meter to read 3.55 at 20 °C, 3.56 at 25 °C.

C.3 Determination of pH of sample

Rinse electrodes free of bitartrate by dipping in H_2O and then in sample. Place electrodes in fresh sample, determine temperature, and read pH to nearest 0.01 unit.

Annex D

Determination of volatile acidity

D.1 Apparatus

- (a) **Steam distillation apparatus** – See Figure 960.16 (see 26.1.32) of the AOAC Manual
- (b) **Cash electric still** – See Figure 964.08 of the AOAC Manual. Consists of outer chamber, inner chamber, trap, 2-way stopcock, electric coil heater, and glass “T” outlet for H₂O. All parts are of Pyrex. Residue in inner chamber after distillation is flushed out automatically by vacuum action when current is shut off. Addition of H₂O through funnel above stopcock gives automatic spray bath to inner chamber, and waste drains through outlet in glass “T”. Two-way stopcock permits introduction of sample, serves as escape vent for CO₂, and allows introduction of wash H₂O.

D.2 Preparation of sample

Remove dissolved CO₂ from ca. 50 L sample by either: placing under low vacuum (H₂O aspirator) 2 min with continuous stirring; or bringing to incipient boiling under air condenser and cooling immediately.

D.3 Determination

- (a) **Steam distillation apparatus** – Add ca 600 L boiled H₂O to outer chamber of still. Pipet 25 mL freshly prepared sample into inner chamber and stopper. Boil H₂O 3 in with sidearm open. Close and distill ca 300 mL into Erlenmeyer. Add 0.5 mL phenolphthalein to distillate and titrate rapidly with 0.1N NaOH until pink persists 15s. express results as g CH₃COOH/100 mL = mL 0.1N NaOH x 0.006 x 4.
- (b) **Cash electric still** – Add H₂O and pipet sample as in (a). Rinse funnel with ca 5 mL H₂O. Distill ca 300 mL into Erlenmeyer. Titrate and express results as in (a). (Disconnect heating coil immediately and empty still by opening drain tube to outer chamber and stopcock to inner tube. Rinse still with two 10 mL-15 mL portions H₂O by adding through funnel; evacuate each portion through drain tube.)

Annex E**Determination of titratable acidity in wines**

Remove CO₂ if present, by either of the following methods:

- (1) Place ca 25 mL sample in a small Erlenmeyer flask and connect to H₂O aspirator. Agitate 1 in under vacuum; or
- (2) Place ca 25 mL sample in a small Erlenmeyer flask, heat to incipient boiling and hold 30 s, swirl, and cool.

Add 1 mL phenolphthalein indicator solution to 200 mL hot, boiled H₂O in 500 mL wide-mouth Erlenmeyer flask. Neutralize to distinct pink. Add 5.00 mL degassed sample and titrate with 0.1 N standardized NaOH to same end point, using well-illuminated white background.

To express titratable acidity as grams of lactic acid per 100 mL of wine,

$$\text{g lactic acid / 100 mL} = \text{mL NaOH} \times \text{normality of NaOH} \times 0.090 \times 100/5$$

Annex F

Determination of total soluble solids

F.1 Apparatus

(a) **Hand refractometer** – With scale reading of 0 °-35 °Brix.

F.2 Standardization of refractometer

Adjust instrument to read n of 1.3330 of 0 % sucrose with H₂O at 20 °.

F.3 Preparation of sample

Bring the sample to a temperature close to 20 °C, then filter to remove it of any undissolved solids

F.4 Determination

Place sufficient amount of sample on the prism of the instrument, taking care that the sample covers the glass surface uniformly. Determine the total soluble solids by direct reading in terms of °Brix.

Annex G

Determination of alcohol by volume from specific gravity

G.1 Distillation of sample

Measure 100 mL original material into 300 mL - 500 mL distillation flask, noting temperature, and add 50 mL water. Attach flask to vertical condenser by means of bent tube, distill almost 100 mL, and dilute to 100 mL at same temperature. (Foaming, which sometimes occurs, especially with young wines, may be prevented with by adding a small amount of antifoam material) For wines that contain an abnormal amount of CH_3COOH , neutralize exactly with 1N NaOH solution before proceeding with distillation (unnecessary for wines of normal taste and odor).

G.2 Calibration

Fill thoroughly cleaned pycnometer with recently distilled water, stopper, and immerse in constant temperature water bath with bath level above graduation mark on pycnometer. After 30 min, remove stopper and with capillary tube adjust until bottom of meniscus is tangent to graduation mark. With small roll of filter paper, dry inside neck of pycnometer, stopper, and immerse in water at room temperature for 15 min. Remove pycnometer, dry, let stand 15 min, and weigh. Empty pycnometer, rinse with acetone, and dry thoroughly in air with suction. Let empty flask come to room temperature, stopper, and weigh.

Weight of water = weight of pycnometer + water – weight of empty pycnometer

G.3 Determination of specific gravity at room temperature

1. Determine weight of sample as in F.2.

Weight of sample = weight of pycnometer + distillate – weight of empty pycnometer

2. Calculate specific gravity as follows:

Specific gravity = $\frac{S}{W}$

where

S is the weight of sample;

W is the weight of water.

G.4 Determination of alcohol

Obtain corresponding % alcohol by volume from Appendix C: Reference Volumes 913.02. AOAC Manual. 16th ed.

Annex H

Determination of total phenols

H.1 Reagents

1. Folin-Denis Reagent – To 750 mL water, add 100 g sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot \text{H}_2\text{O}$), 20 g phosphomolybdic acid ($\text{H}_3\text{P}(\text{Mo}_3\text{O}_{10})_4 \times \text{H}_2\text{O}$) and 50 mL phosphoric acid (H_3PO_4). Reflux for 2 hours, cool and dilute to 1 liter. Store in an amber bottle.
2. Sodium carbonate saturated solution – To each 100 mL water, add 35 g anhydrous sodium carbonate (Na_2CO_3), dissolve at 70-80°C and let cool overnight. Seed supersaturated solution with crystal of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ and after crystallization, filter through glass wool.
3. Tannic acid standard solution – 0.1 mg/mL. Dissolve 100 mg tannic acid in 1 L water. Prepare fresh solution for each determination. Gallic acid can also be used.

H.2 Preparation of standard curve

Pipet 0 mL-10 mL aliquots standard tannic acid solution into 100-mL volumetric flasks containing 75 mL water. Add 5 mL Folin-Denis reagent and 10 mL Na_2CO_3 solution and dilute to volume with water. Mix well and determine the absorbance after 30 minutes at 760 nm. Plot absorbance against mg tannic acid/mL.

H.3 Determination

Using 1 mL of sample, determine absorbance as in the preparation of the standard curve and obtain mg tannic acid/100 mL for the standard curve. If absorbance is too great (>0.7), repeat determination on 1 + 4 dilution of sample.

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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FORMULATING BODY
Development of Standards for Selected Ethnic Food Products – Phase II
Standards for Sugar Cane Wine (Basi)

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